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Thymopoietin, a Thymic Polypeptide, Regulates Nicotinic α-Bungarotoxin Sites in Chromaffin Cells in Culture

M. QUIK, R. AFAR, S. GEERTSEN, T. AUDHYA, G. GOLDSTEIN, and J.-M. TRIFARÓ

Department of Pharmacology, McGill University, Montreal, Quebec H3G 1Y6, Canada (M.Q., R.A., S.G.), Immunobiology Research Institute, Annandale, New Jersey 08801-0999 (T.A., G.G.), and Secretory Process Research Program, Department of Pharmacology, University of Ottawa, Ottawa, Ontario K1H 8M5 Canada (J.-M.T.)

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SUMMARY

The identity of the neuronal nicotinic α-bungarotoxin (α-BGT) site, which appears to be distinct from the functional nicotinic receptor, is unclear. Recent work in our laboratory has shown that the thymus-derived polypeptide thymopoietin potently and specifically interacts at the nicotinic α-BGT site in brain. The present results show that thymopoietin also interferes with the binding of ^{125}I-α-BGT to chromaffin cells in culture; a dose-dependent inhibition in binding was observed, with an IC_{50} of 10^{-8} M. To assess the long term effect(s) of thymopoietin in nervous tissue, chromaffin cells were exposed to the polypeptide for varying periods of time. Incubation of the cells in culture with thymopoietin (10^{-9} to 3×10^{-7} M) for 2 to 7 days resulted in an approximate 3-fold increase in α-BGT binding. Saturation analysis indicated this was due to an increase in the B_{max}. The thymopoietin-induced increase in binding could be reversed with nicotine; thus, the sites can be regulated by a nicotinic receptor ligand. Although thymopoietin potently interacted at the nicotinic α-BGT receptor, nicotinic receptor responsiveness was not affected after short or long term exposure to the peptide. Neither basal nor nicotinic receptor-stimulated tyrosine hydroxylase activity was altered by thymopoietin. As well, resting and acetylcholine-evoked noradrenaline release remained similar to control after exposure of the cells to the polypeptide. These results indicate that the thymic polypeptide thymopoietin specifically interacts with the nicotinic α-BGT receptor population and, furthermore, can regulate the toxin binding sites in chromaffin cells in culture.

On the basis of current evidence, several distinct populations of nAchR appear to exist (1–8). One of these is the nAchR present at the neuromuscular junction and in electric tissue. Another is the nAchR localized in nervous tissue, which may exist as several subtypes composed of various forms of an α- and β-subunit (possibly as $\alpha_2\beta_2$); as well, there is also the neuronal α-BGT site. The neuronal nAchR binds nicotinic cholinergic agonists with high affinity but not α-BGT (9, 10); this nicotinic receptor is currently thought to mediate functional responses, an assumption based on a number of different criteria including regional distribution of the binding site in brain and receptor regulatory properties. The α-BGT site, on the other hand, appears to represent a class of cholinoceptors that binds α-BGT with high affinity but nicotine only with low affinity (9, 11, 12). The functional identity of the α-BGT site is not yet known, inasmuch as the toxin does not block cholinergic responses at most neuronal synapses (13, 14). However, a link with a nicotinic cholinergic recognition site is suggested from the receptor binding studies just mentioned, from the observation that α-BGT blocks cholinergic responses in certain brain regions including the inferior colliculus (15) and cerebellum (16), and from the finding that antibodies against the electroplax nAchR cross-react with the α-BGT binding component in neuronal tissues (17, 18). Although the role of the α-BGT site is not clear, it has been implicated in circadian rhythms, hormonal regulation, and neuronal maintenance and/or growth (19–21). Thus, the neuronal α-BGT binding component may represent a nicotinic receptor subtype involved in functions that may or may not be associated with synaptic transmission, that is, ion translocation.

In addition to the above hypothesis, it is also possible that ligands other than acetylcholine might interact at the α-BGT recognition site. Previous work (22) had indicated the existence of an endogenous ligand for the neuronal α-BGT site, although its identity remained elusive. More recently, studies from our laboratory have shown that thymopoietin potently competed with α-BGT at its binding site in brain membranes (23, 24). Thymopoietin inhibited α-BGT binding in the nanomolar range, whereas nAchR ligands such as nicotine and *d*-tubocurarine were effective only at micromolar concentrations. Furthermore, thymopoietin specifically interacted with the α-BGT receptor population; it did not alter binding of [^{3}H]nicotine or

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ABBREVIATIONS: nAchR, nicotinic acetylcholine receptor; α-BGT, α-bungarotoxin.

[^{3}H]methylcarbachol to the nAchR nor [^{3}H]quinuclidinylbenzilate binding to the muscarinic receptor. Because thymopoietin has been identified in the circulation (25) and in central nervous system tissue (26), this polypeptide may represent an endogenous ligand at the α-BGT site in brain.

Thymopoietin, a 49-amino acid polypeptide isolated from thymus, is involved in immune responsiveness; it affects differentiation of T and B cells in addition to having other regulatory properties (27). As well, thymopoietin has been shown to inhibit ^{125}I-α-BGT binding to electroplax nAchR (28) and, furthermore, to alter function at the neuromuscular junction (29, 30), possibly through a desensitization of the nAchR (31). Because the peptide has the potential to modulate nAchR activity in muscle, experiments were done to assess its effect(s) in a neuronal system. The model system selected was adrenal chromaffin cells in culture, because these cells are of neural crest origin and have both a nAchR and a nicotinic α-BGT binding site, which appear to be distinct. This conclusion was based on the observation that the toxin did not block nAchR-mediated function (32–34) and on findings that showed that the nAchR population and the α-BGT receptors were not consistently altered in parallel after exposure to nicotinic agonists and antagonists, as well as other agents (35–37). Experiments were first carried out to determine whether thymopoietin could interact with the α-BGT site in chromaffin cells in culture; subsequent studies determined whether the polypeptide could regulate the toxin binding sites and/or nAchR function in the cells.

Experimental Procedures

Materials. Thymopoietin was isolated and purified from bovine thymus (38, 39). Purity of thymopoietin was 94–96%, as determined by polyacrylamide gel electrophoresis, isoelectric focussing (pI 6.82), and fast protein liquid chromatography on a reverse phase column. Gas phase sequencing of several batches yielded a single N-terminal sequence. With C-terminal analysis some minor heterogeneity was observed between batches, and this was attributable to variable carboxypeptidase cleavage during isolation of the polypeptide. As an index of potency of the polypeptide, thymocytes responded to thymopoietin with an EC_{50} of approximately 0.2 nM (39).

^{125}I-α-BGT (10–20 μCi/μg), [^{3}H]tyrosine (L-[*ring*-3,5-^{3}H]tyrosine, 40–60 Ci/mmol), and [^{3}H]noradrenaline (*l*-[*ring*-2,5,6-^{3}H]noradrenaline, 43.7 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Dihydro-β-erythroidine was a generous gift from Merck, Sharp and Dohme (Kirkland, Quebec, Canada). Nicotine and *d*-tubocurarine were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from standard commercial sources.

^{125}I-α-BGT binding to chromaffin cells in culture. Bovine adrenal glands were placed in cold sterile Locke's solution. The cortex was removed, and the medulla was perfused with Ca^{2+}-Mg^{2+}-free Locke's solution. The chromaffin cells were isolated, purified, and cultured according to the method of Trifaró and Lee (32). The binding of α-BGT to 7- to 14-day-old chromaffin cell cultures (10^6 cells/dish) was determined as previously described (35). During the last 2–7 days in culture, the cells were incubated without or with the indicated agents. To remove any residual drugs before the α-BGT binding assay, the medium was removed and the cells were washed extensively with Locke's buffer. This involved three washes with 2 ml of Locke's buffer over a period of 30 min, with two further washes with 2 ml of Locke's buffer, each with a 30-min incubation at 37°. This was followed by a 60-min preincubation of the cell cultures at 37° in the presence or absence of the indicated drug. ^{125}I-α-BGT (1.5 nM) was then added to each plate for a 90-min incubation at 37°. The cells were subsequently washed six times with 2-ml aliquots of Locke's buffer over a 60-min period to remove excess radiolabeled α-BGT.

[^{3}H]Noradrenaline release studies in chromaffin cells in culture. Seven- to 9-day-old chromaffin cell cultures were incubated in the absence or presence of thymopoietin for the indicated number of days. On the day of the experiment, each culture well (5×10^5 cells/well) was washed as described for the ^{125}I-α-BGT binding assay. The cells were then incubated for 1 hr at 37° with 250 μl of amino acid-free Dulbecco's modified Eagle's medium containing 0.25 μCi of [^{3}H]noradrenaline (0.2×10^{-7} M). Following the loading phase, cell cultures were washed seven times with regular Locke's buffer over a 1-hr period. Basal release was determined for a 3-min incubation period before stimulated release; release of [^{3}H]noradrenaline in the presence acetylcholine was measured over three consecutive 3-min stimulation periods, followed by one more 3-min period of nonstimulated release of the radiolabeled compound. Ice-cold trichloroacetic acid (10%) was added to lyse the cells and determine intracellular [^{3}H]noradrenaline content. Total uptake of [^{3}H]noradrenaline is represented by the sum of radioactivity measured in the acid extract and the radioactivity secreted under basal and stimulating conditions. Release was expressed as percentage of total [^{3}H]noradrenaline originally taken up per 5×10^5 cells.

Tyrosine hydroxylase assay. Tyrosine hydroxylase activity was measured according to the method of Nagatsu *et al.* (40), as previously described (34). The cells in culture (0.5×10^6 cells/well) were rinsed with 1.0 ml of 50 mM phosphate buffer, pH 7.4, containing 0.9% NaCl and were then quickly washed with 1.0 ml of 5.0 mM Tris·HCl containing 0.1% Triton X-100, pH 7.4. A 700-μl aliquot of the same buffer was then added to the culture dish and the cells were scraped from the plate. This suspension was homogenized and a 60-μl aliquot was used for assay. The concentration of L-tyrosine was 10 μM and of cofactor (6,7-dimethyl-5,6,7,8-tetrahydropteridine-HCl) was 100 μM. The time of incubation was 15 min.

DNA determinations. To determine the DNA content of the chromaffin cell samples, 0.5 ml of 1.8 N trichloroacetic acid was added to the cells that had previously been suspended in 1 ml of Locke's buffer. The samples were prepared as previously described (35) and the DNA content was determined according to the method of Setaro and Morley (41).

Statistics. Statistical comparisons were done using Student's *t* test.

Protein determinations. Protein was determined using the method of Lowry *et al.* (42), with bovine serum albumin as standard.

Results

Effect of thymopoietin on α-BGT binding to chromaffin cells in culture. To determine whether thymopoietin could interact with the α-BGT binding site on adrenal medullary cells in culture, cells were preincubated with varying concentrations of the polypeptide for 60 min. Fig. 1 shows that incubation with thymopoietin resulted in a dose-dependent decrease in toxin binding, with an IC_{50} of 12 nM. The IC_{50} for α-BGT was 1.1 nM, whereas the nicotinic receptor ligands had a much lower potency, with IC_{50} values of 2400, 4500, and 42,000 nM for *d*-tubocurarine, nicotine and dihydro-β-erythroidine, respectively.

Effect of long term exposure to thymopoietin on ^{125}I-α-BGT binding to chromaffin cells in culture. To test whether thymopoietin could regulate the α-BGT receptors, the cells in culture were exposed to varying concentrations of the peptide over several days (Fig. 2). An increase in ^{125}I-α-BGT binding was observed with increasing concentrations and time. After a 2-day exposure period to the polypeptide, α-BGT binding was enhanced only with the highest concentration (3×10^{-7} M) of thymopoietin; higher concentrations were not used due to the limited availability of the peptide. Four- or 7-day expo-

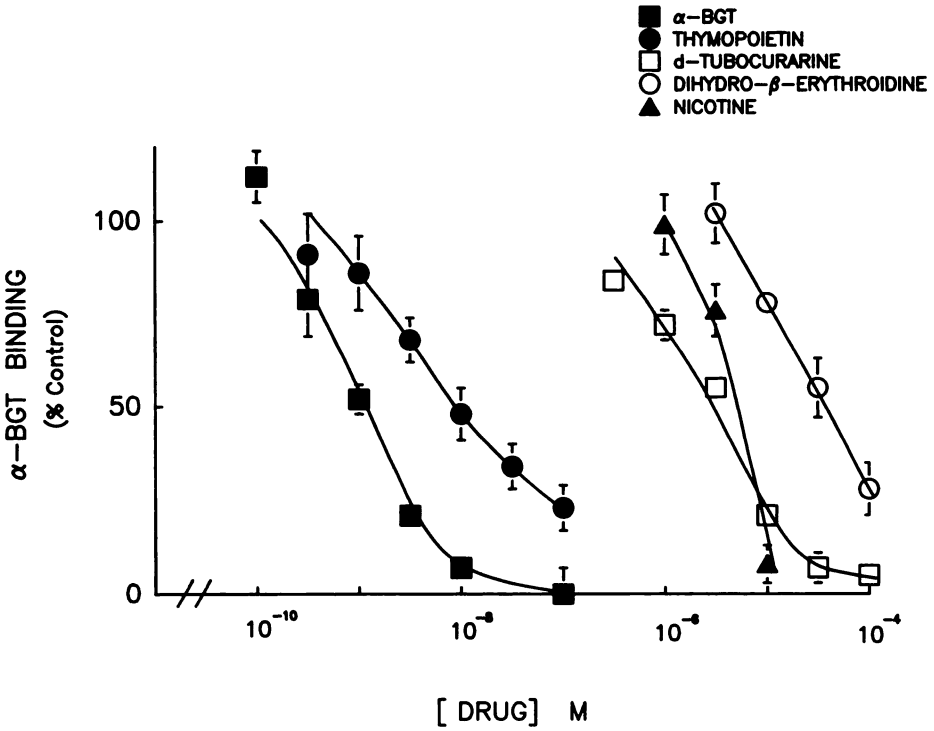


Fig. 1. The effect of thymopoietin and nicotinic cholinergic ligands on ^{125}I-α-BGT binding to chromaffin cells. Cells in culture were preincubated for 60 min in the absence or presence of varying concentrations of the indicated compounds. This was followed by a 90-min incubation with 1.5 nM ^{125}I-α-BGT. The binding assay was terminated as described. Each value represents the mean ± standard error of 3 to 15 culture dishes.

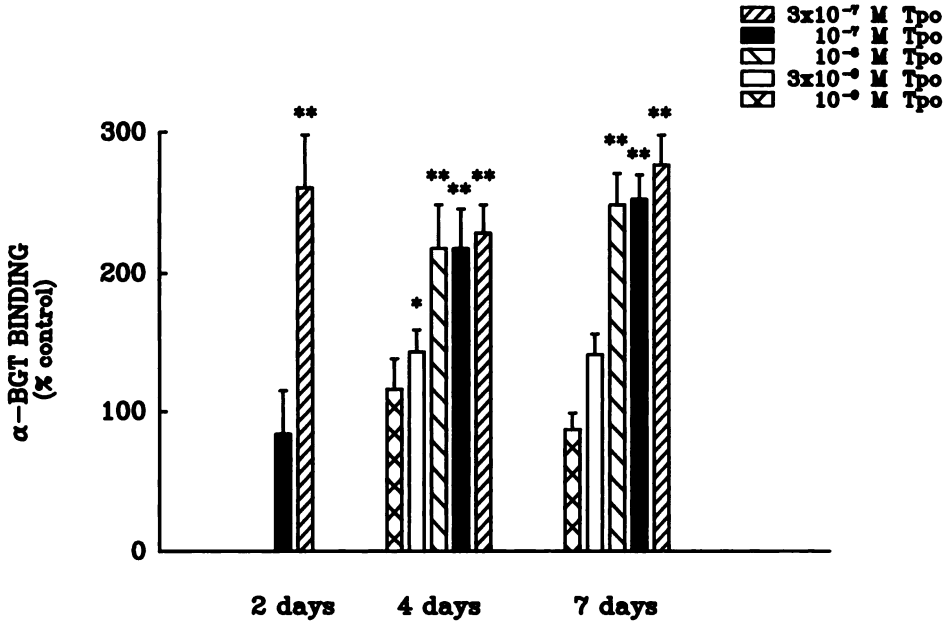
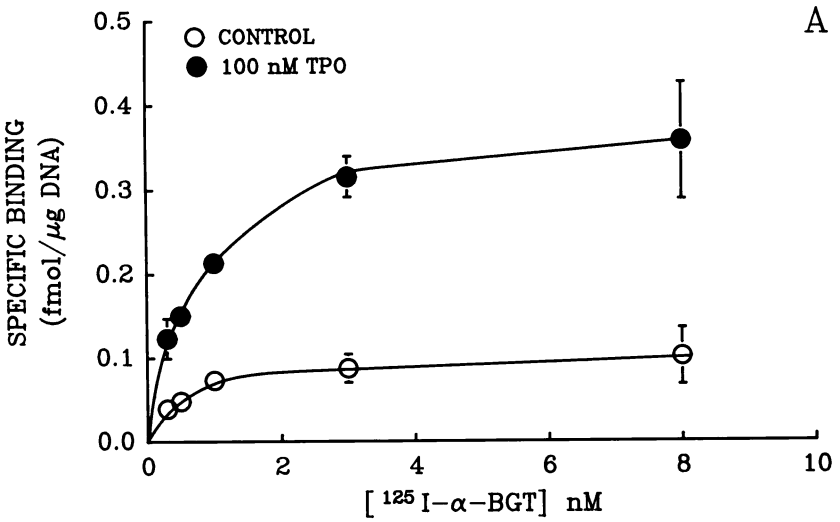


Fig. 2. The effect of long term exposure to thymopoietin (*Tpo*) on ^{125}I-α-BGT binding to chromaffin cells. Cells in culture were exposed to the indicated concentrations of thymopoietin for 2 to 7 days. The cells were then extensively washed to remove thymopoietin and the ^{125}I-α-BGT binding assay was done as described. Control binding was 0.074 ± 0.018 fmol/μg of DNA (18 determinations). Each *bar* represents the mean ± standard error of 3 to 15 culture dishes. Significance of difference from control: $^{*}p < 0.05$; $^{**}p < 0.001$.

sure to thymopoietin led to similar results, with a maximal increase in binding occurring with 10^{-8} M polypeptide. As an index of cell viability, the DNA content of the cultures was determined; no changes were observed at any of the concentrations or time points, as compared with the control culture.

The increase in α-BGT binding in response to thymopoietin appeared to be due to an increase in the number of receptors (Fig. 3); Scatchard analysis yielded a B_{max} of 0.11 and 0.38 fmol/μg of DNA for the control and thymopoietin-treated cultures, respectively, whereas the K_D was 0.55 nM for the controls and 0.71 nM for the cultures exposed to the polypeptide. No difference in the DNA content of the cultures was observed between the control and treated groups.

Chromaffin cells were preincubated for 7 days in the absence or presence of 10^{-4} M nicotine and/or varying concentrations of thymopoietin (Fig. 4). Thymopoietin on its own resulted in up to a 3-fold increase in ^{125}I-α-BGT binding at the highest concentration of the polypeptide used. Nicotine per se resulted in a decrease in α-BGT binding; the agonist also completely prevented the increase in α-BGT sites induced by the thymic polypeptide. In this experiment, exposure of the cells to 10^{-8} M thymopoietin resulted in a less than maximal increase in binding; however, a maximal increase in toxin binding was observed with 10^{-8} M thymopoietin in the experiment depicted in Fig. 2. Because the results in Fig. 2 are the combined data from three experiments, these results may represent the situation more



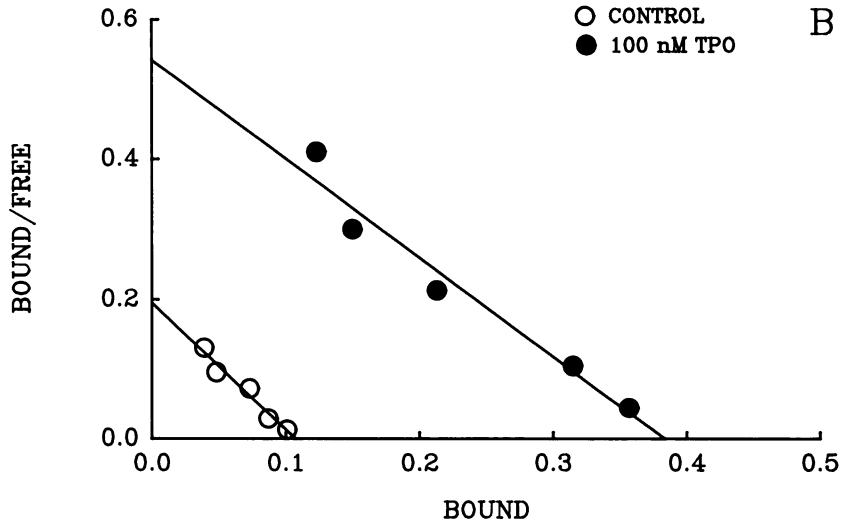


Fig. 3. Saturation curve (A) and Scatchard analysis (B) of α-BGT binding to adrenal chromaffin cells cultured for 4 days in the absence or presence of 10^{-7} M thymopoietin (*TPO*). Each value in A represents the mean ± standard error of 8 to 10 culture dishes; where the standard error is not depicted, it was within the *symbol*. For the Scatchard plot (B), *BOUND/FREE* on the *y*-axis is expressed as fmol/μg of DNA/nM and *BOUND* on the *x*-axis as fmol/μg of DNA. The results are representative of three separate experiments.

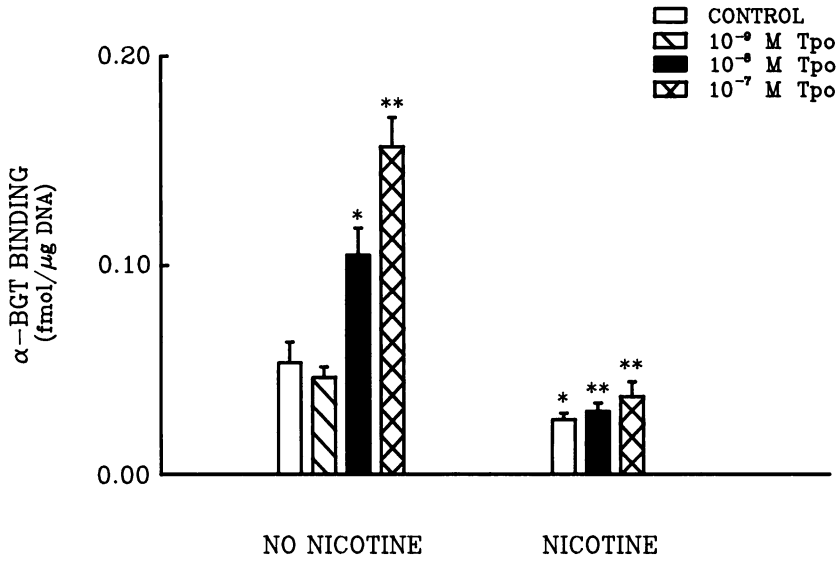


Fig. 4. Effect of nicotine on the thymopoietin-induced increase in ^{125}I-α-BGT binding to chromaffin cells. Cells in culture were incubated in the absence or presence of 10^{-4} M nicotine, without or with the indicated concentrations of thymopoietin (*Tpo*), for 6 days. The cells were then extensively washed to remove the drugs and ^{125}I-α-BGT binding was measured. Each *bar* represents the mean ± standard error of four or five culture dishes. Significance of difference from *CONTROL* in the absence of thymopoietin: $^{*}p < 0.05$; $^{**}p < 0.001$.

accurately. The reasons for the observed variability in binding in response to thymopoietin are not clear; however, such variations in α-BGT binding have also been observed in previous studies with chromaffin cells in culture (36). They may arise as a result of variability in the source of the cells (different groups of animals) from culture to culture, as well as differences in batches of fetal calf serum, medium, and other materials used in the culture process.

Effect of thymopoietin on nAchR-mediated responses in chromaffin cells in culture. To determine whether thymopoietin could alter nicotinic sensitivity in chromaffin cells in culture, its effects were tested on acetylcholine-evoked [^{3}H] noradrenaline release. Preincubation of the cells with 10^{-7} M thymopoietin for 10 min, as well as the presence of the polypeptide during the release periods, had no effect on basal or acetylcholine-evoked [^{3}H]noradrenaline release (Fig. 5). Similar results were obtained with preincubation periods ranging from 5 to 30 min and thymopoietin concentrations from 10^{-8} to 10^{-6} M. In the present experiment, incubation with 3×10^{-5} M acetylcholine resulted in a half-maximal release of [^{3}H] noradrenaline. When 10^{-5} or 10^{-4} M acetylcholine (to result in a minimal or maximal catecholamine release, respectively) was used to stimulate [^{3}H]noradrenaline release, the pattern of release was not different for control or thymopoietin-treated cultures.

Basal and acetylcholine-stimulated release of [^{3}H]noradrenaline were also measured from cells in culture that had been exposed to 10^{-7} M thymopoietin over a longer time period (4 days). No differences in the release pattern of radiolabeled catecholamines were observed for control as compared with treated cultures (Fig. 5). Similar results were obtained whether 10^{-5}, 3×10^{-5}, or 10^{-4} M acetylcholine was used to stimulate

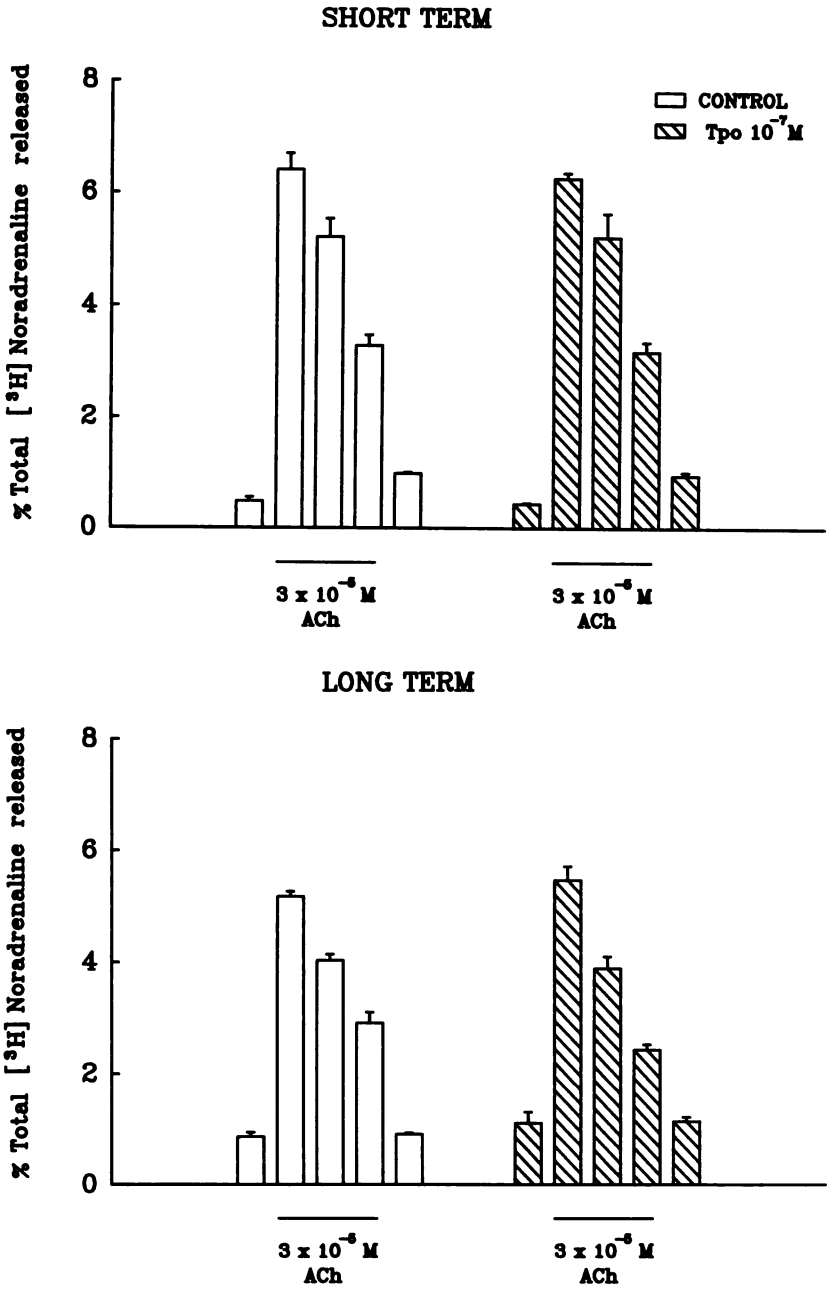


Fig. 5. Effect of thymopoietin on basal and acetylcholine-evoked [^{3}H]noradrenaline release from chromaffin cells. Cells in culture were preincubated for 10 min (*SHORT TERM*) in the absence or presence of 10^{-7} M thymopoietin (*Tpo*). In the *bottom* (*LONG TERM*), the cells in culture were exposed to 10^{-7} M thymopoietin for 4 days; the thymopoietin was removed by washing of the cells as described. Release of [^{3}H]noradrenaline was then determined. Each *vertical bar* represents a 3-min release period. The *horizontal bar* indicates the duration of exposure to acetylcholine (*Ach*). Each value represents the mean ± standard error of four to eight culture wells.

[^{3}H]noradrenaline release. As well, varying the thymopoietin concentration (10^{-8} to 10^{-6} M) or time of exposure to the polypeptide (2 to 7 days) yielded no differences between the control and treated cultures.

As another index of nicotinic sensitivity, the effect of thymopoietin was determined on basal and carbachol-evoked tyrosine hydroxylase activity in chromaffin cells in culture (Table 1). Cells were exposed to 10^{-7} M thymopoietin for 2 or 5 days. Basal tyrosine hydroxylase activity remained similar to control under these conditions. Incubation with carbachol (10^{-4} M) resulted in a 41 and 45% increase in enzyme activity after a 2- and 5-day period, respectively; this response was completely blocked by *d*-tubocurarine at a concentration of 5×10^{-5} M and was not significantly altered in the presence of thymopoietin.

Discussion

The present results show that thymopoietin, a polypeptide isolated from thymus, inhibited ^{125}I-α-BGT binding to chromaffin cells in culture with an IC_{50} in the nanomolar range (12 nM), a value similar to that observed for thymopoietin inhibition of ^{125}I-α-BGT binding to brain (23, 24). Adrenal medullary chromaffin cells in culture, which have been used extensively as a model for neuronal cells, may therefore prove useful in determining the role of thymopoietin in nervous tissue. In the present studies, in agreement with established work (9, 11, 12), nicotinic receptor ligands prevented binding of radiolabeled toxin to chromaffin cells in short term competition experiments at concentrations in the range of 10^{-6} to 10^{-4} M. These results thus show that, except for the α-toxin itself, thymopoietin is the most potent agent to interact at the α-BGT binding site.

Thymopoietin, in addition to its presence in the thymus, has been identified in serum at a concentration of approximately 3 nM (25); thus, circulating polypeptide levels could potentially interact with the α-BGT sites in adrenal medulla. In addition, thymopoietin-like immunoreactivity has been detected in brain and spinal cord extracts in concentrations ranging from 1 to 10 nM (26); a neural origin of this thymopoietin was suggested from findings that demonstrate the presence of the polypeptide in neuronal but not glial cells. The observation that thymopoietin is present endogenously in mammals, together with the finding that it is the most potent compound to interact at the α-BGT site except for α-BGT itself, suggests that thymopoietin may represent an endogenous ligand for the α-toxin binding site.

Previous work (23, 24) had shown that thymopoietin did not affect [^{3}H]nicotine and [^{3}H]methylcarbachol binding to brain membranes, suggesting that the polypeptide specifically interacted with the nicotinic α-BGT receptor population. To determine whether thymopoietin exhibited a similar selectivity in chromaffin cells in culture, the effect of the polypeptide was determined on a functional response mediated by the nAchR, that is, acetylcholine-evoked [^{3}H]noradrenaline release. Neither resting nor evoked catecholamine release was affected after short term exposure (minutes) to the polypeptide, suggesting that in this particular neuronal preparation thymopoietin again interacts only at the nicotinic α-BGT site. Because the α-BGT sites were measured using a binding assay, we attempted to measure the nAchR in the chromaffin cells in culture using a similar approach. However, specific binding of [^{3}H]nicotine or [^{3}H]methylcarbachol could not be reproducibly obtained in our hands, although specific binding of these radioligands was readily observed in brain. This could possibly be attributed to the following factors: binding of [^{3}H]nicotine to the receptor is rapidly reversible, the affinity of the radioligand for the binding site is only 20 nM, and/or the density of nAchR binding sites in chromaffin cells in culture is fairly low (37).

Because thymopoietin interacted at the α-BGT site in chromaffin cells in culture, experiments were subsequently done to determine whether the polypeptide could regulate the α-toxin sites in these cells. Many neurotransmitter and/or neuromodulator compounds have the potential to up- or down-regulate their respective receptors after long term exposure to these agents (43). Thymopoietin resulted in up to a 3-fold increase in α-BGT binding after 4 to 7 days of incubation with a concentration of the polypeptide as low as 10^{-8} M. This correlates well with the concentration of thymopoietin required to prevent α-BGT from acting at its binding site in the short term experiments (IC_{50} = 12 nM). Saturation analysis indicated that the increase in ^{125}I-α-BGT binding in the culture in response to the polypeptide was due to an increase in the maximal number of binding sites. Previous work had shown that in chromaffin cells in culture up-regulation of α-BGT sites by antagonists and other agents could be reversed by simultaneous incubation of the cells with nicotine (36). The thymopoietin-induced increase in α-BGT binding was also reversed in the presence of nicotine. This suggests the sites still exhibit the characteristics of a nicotinic-binding protein; however, the nature of the relationship between the α-BGT, thymopoietin, and nicotinic recognition sites remains to be determined.

In line with the observation that thymopoietin did not affect short term responses mediated through a nAchR, long term (days) exposure to thymopoietin did not modify nAchR-mediated responses in chromaffin cells in culture. As an index of nAchR-mediated sensitivity, the effect of thymopoietin was determined on the carbachol-mediated increase in tyrosine hydroxylase activity. Previous work had shown that the carbachol-induced increase in enzyme activity was blocked by the nicotinic antagonist *d*-tubocurarine but not by α-BGT (34), studies that further confirmed that the functional nAchR and the α-BGT binding site were distinct in chromaffin cells (32–37). The present experiments show that thymopoietin did not affect nAchR-mediated tyrosine hydroxylase activity; as well,

TABLE 1

Effect of thymopoietin on basal and stimulated tyrosine hydroxylase activity in chromaffin cells in culture

After 7 days in culture, 5×10^{-6} M *d*-tubocurarine or 10^{-7} M thymopoietin was added to the cells in culture. This was followed by addition of 10^{-4} M carbachol, as indicated. After 2 or 5 more days of incubation, the medium was removed from the cells and the cultures were washed as described. The cells were then scraped from the dish in 700 μl of 5 mM Tris·HCl containing 0.1% Triton X-100; the enzyme activity was determined in a 60-μl aliquot. Control values on day 2 and 5, respectively, were 4.22 ± 0.19 and 5.07 ± 0.14 nmol of β-3,4-dihydroxyphenylalanine/hr/culture. Each value represents the mean ± standard error of 3 to 11 cultures.

Group	Drug	Tyrosine hydroxylase activity	
		2 days	5 days
		% of control	
Control	None	100 ± 5	100 ± 3
	Carbachol	141 ± 5[a]	145 ± 14[b]
Thymopoietin	None	100 ± 6	106 ± 4
	Carbachol	134 ± 3[a]	142 ± 8[a]
d-Tubocurarine	None	93 ± 4	79 ± 12
	Carbachol	88 ± 1	101 ± 14

[a] Significantly different from control in the absence of carbachol, $p < 0.01$.
[b] $p < 0.05$.

the polypeptide did not alter acetylcholine-evoked [^{3}H]noradrenaline release from the cells in culture. Thus, this further demonstrates the specificity of thymopoietin for the α-BGT receptor population.

A question that arises is whether thymopoietin exhibits the properties of an agonist or antagonist at the α-BGT site. It is difficult to come to any conclusions regarding this matter from the present results. Although in many systems agonists result in long term receptor down-regulation (43), there are exceptions to this rule. For instance, the agonist nicotine can result in either an up- or down-regulation of the α-BGT binding sites; administration of nicotine resulted in an enhanced number of sites in brain and TE671 cells (44–46), whereas no change or a decrease was observed in chromaffin cells in culture (36, 47).

The present studies show that thymopoietin affects the α-BGT binding sites in both the short and long term experiments at nanomolar concentrations. On the other hand, nicotinic receptor ligands alter α-BGT binding either in the competition binding studies or after longer periods of exposure only at concentrations in the micromolar range (35, 36, 44). These results are in contrast to the effects of thymopoietin and nicotinic ligands at the functional receptor. nAchR-mediated responsiveness (catecholamine release or tyrosine hydroxylase activity) was not modified after either short or long term exposure of the chromaffin cells to concentrations of thymopoietin as high as 3×10^{-7} M; nicotinic ligands, on the other hand, produce maximal effects (either activation or blockade) at concentrations ranging from 10^{-7} to 10^{-4} M (32, 33, 48). These studies, thus, indicate that thymopoietin preferentially interacts at the α-BGT sites, without affecting the functional nAchR. Moreover, thymopoietin is shown to regulate the α-BGT sites in the cells in culture. These observations suggest that thymopoietin may be a ligand at the α-BGT site. Experiments are currently in progress to elucidate the functional consequences of the interaction of thymopoietin with the α-BGT site.

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Send reprint requests to: M. Quik, Department of Pharmacology, McGill University, McIntyre Medical Building, 3655 Drummond St., Montreal, Quebec H3G 1Y6, Canada.